

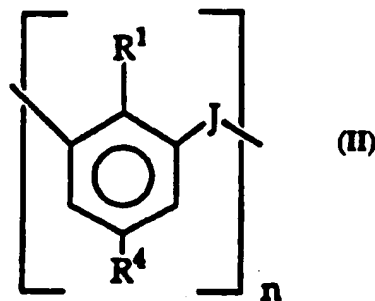
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(54) Title: CALIXARENE CONJUGATES USEFUL AS MRI AND CT DIAGNOSTIC IMAGING AGENTS

(57) Abstract

Calixarene conjugates useful for imaging, particularly magnetic resonance imaging (MRI) and computed tomography (CT) are described. Said calixarene conjugates comprise (i) a calixarene backbone, and (ii) at least one imaging moiety linked thereto, and may be of formula (II) wherein at least one of the R¹ and R⁴ substituents comprises an imaging moiety, the remaining R¹ and R⁴ substituents are spectator groups, J is an ortho-linker, and n is an integer from 4 to 8. Imaging moieties useful for CT imaging include those comprising two or more iodine atoms. Imaging moieties useful for MRI include (i) organic moieties comprising four or more fluorine atoms; (ii) nitroxyl spin labeled moieties; and (iii) metal chelate moieties.



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5 CALIXARENE CONJUGATES USEFUL AS MRI AND CT DIAGNOSTIC
 IMAGING AGENTS

Description

10

Technical Field

 This invention relates to diagnostic imaging agents which are useful for magnetic resonance imaging (MRI) and computed tomography (CT).

15

Background

 Medical diagnostic imaging has evolved as an important non-invasive tool for the evaluation of pathological and physiological processes. Presently, nuclear magnetic resonance imaging (MRI) and computerized tomography (CT) are two of the most widely used imaging modalities. Although both MRI and CT can be performed without the administration of contrast agents, the ability of many contrast agents to enhance the visualization of internal tissues and organs has resulted in their widespread use.

 Proton MRI is based on the principle that the concentration and relaxation characteristics of protons in tissues and organs can influence the intensity of a magnetic resonance image. Contrast agents which are useful for proton MRI effect a change in the relaxation characteristics of protons which can result in image enhancement and improved soft-tissue differentiation. Different classes of proton MR imaging agents include paramagnetic metal chelates and nitroxyl spin labelled compounds.

Two commercially available paramagnetic chelates
5 are PROHANCE (Squibb Diagnostics, Princeton, New
Jersey) and MAGNEVIST (Berlex, Wayne, New Jersey).
(See also, *inter alia*, H.J. Weinman et al., Am. J.
Roentgenol. 142:619-624, 1984; M.-M. Le Mignon, et
al., Investigative Radiology 25:933, 1990; A. D.
10 Sherry et al., U.S. Patent No. 5,316,757, issued 1994;
and A. D. Sherry et al., PCT Application No. WO
92/08725, published 1992.)

Examples of nitroxyl spin labeled compounds are
15 described by R. C. Brasch et al., Radiology 147:773-
779, 1983; G. M. Rosen, U.S. Patent No. 4,834,964,
issued 1989; G. M. Rosen et al., U.S. Patent No.
5,104,641, issued 1992; J. F. W. Keana et al., U.S.
Patent No. 4,863,717 issued 1989; G. M. Rosen, U.S.
20 Patent No. 5,256,397 issued 1993; Y. Berchadsky et
al., U.S. Patent No. 5,006,663, issued 1991; and I.B.
Leunback, PCT Application No. WO 90/00904, published
1990.

25 Fluorine (^{19}F) MRI is also in the early stages of
development. Because of the 100% natural abundance of
 ^{19}F and the complete absence of biological background,
 ^{19}F MRI promises to be an important diagnostic imaging
tool of the future. Fluorine-containing imaging agents
30 include perfluoro-tert-butyl containing organic
compounds (W. J. Rogers, Jr., et al., U.S. Patent No's
5,116,599 issued 1992, 5,234,680 issued 1993, and
5,324,504 issued 1994) and fluoro-substituted benzene
derivatives (P. Blaszkiewicz et al., U.S. Patent No.
35 5,130,119 issued 1992.)

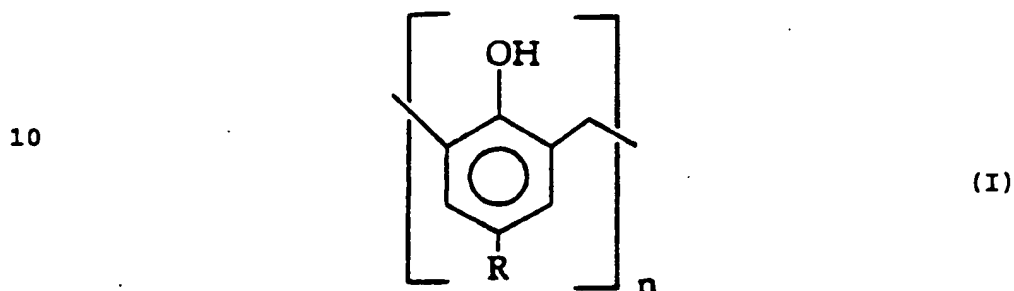
CT is based on the principle that various
substances effect different degrees of attenuation of

an X-ray beam. Contrast agents useful for CT usually
5 contain atoms which are electron dense, such as
bromine or iodine, and are efficient attenuators of X-
ray radiation. By far the most common CT agents are
monomeric or dimeric iodinated benzene rings with
various pendent groups such as ORAGRAFIN, CHOLOGRAFIN
10 and RENOGRAFIN (Squibb Diagnostics, Princeton, New
Jersey). One important advance in the use of iodine-
containing CT agents has been the development of non-
ionic contrast agents, such as the ones described by
M. T. Kneller et al., PCT Application No. WO 93/10825
15 published 1993.

The usefulness and efficiency of chemical
compounds as contrast agents depends on their ability
to exhibit a predictable and desirable biodistribution
20 and metabolism in vivo. Their behavior in vivo
depends on parameters such as molecular weight,
charge, osmolality, hydrophobicity, partition
coefficient, susceptibility to metabolic breakdown,
and tissue or organ targeting efficiency. In order to
25 improve their solubility and biodistribution, many
contrast agents are used in conjunction with delivery
systems such as emulsions, liposomes, and
microparticles. Others are combined with polymeric
systems which allow complex contrast agents to be
30 designed with specific molecular weight, charge and
targeting characteristics. For example, contrast
agents can be conjugated to dense star polymers (see,
for example, Tomalia et al., U.S. Patent No.
5,338,532, issued 1994) or amino acid polymers (D.
35 Meyer, et al., PCT WO 93/10824, published 1993).

The present invention relates to novel CT and MRI
agents which are in the form of calixarene conjugates.

Calixarenes are macrocycles comprising phenolic units
5 ortho-linked by methylene bridges, as represented by
the following formula:



15 wherein n is typically 4, 5, 6, 7, or 8, and more
commonly 4, 6, or 8. Calixarenes are commonly
referred to as calix(n)arenes wherein n refers to the
number of phenolic units. As denoted herein, the
phenolic -OH group occupies the 1-position, and the
20 substituent -R group occupies the 4-position.
Although different conformations are possible
depending on the type and degree of derivatization,
calixarenes are often described as being basket- or
cup-shaped, with a larger diameter upper rim comprised
25 of substituents at the 4-positions and a smaller
diameter lower rim comprised of substituents at the
1-positions.

30 Calixarenes were first discovered in the 1940's
(see, *inter alia*, J.B. Niederl et al., J. Am. Chem.
Soc., 62:2512-2514, 1940). A variety of calixarenes
and calixarene derivatives have been prepared and
characterized (see, *inter alia*, C.D. Gutsche,
Calixarenes, 1989, Royal Society of Chemistry,
35 Cambridge, UK; and Z. Asfari et al., Jansen 24 Chimica
Acta, 10(1):3-10, 1992) and include, for example,
alternative substituents at the 1- and/or 4-positions,

and alternative ortho-linkages, such as $-(C=O)-$,
5 $-CH_2CH_2-$, and $-CH(CH_3)-$.

Calixarenes have found use in catalysis
(polymerization accelerators), transport and
extraction of metallic cations (cesium ion extraction,
10 metal ion sequestrants), and in modifying the chemical
properties of polymers, drugs, and dyes (see, inter
alia, Z. Asfari et al., *supra*; and W.I. Hwang et al.,
PCT Application No. WO 94/03164 published 1994).

15 Recently, Bakker et al. (*J. Org. Chem.*, 59:972-
976, 1994) have disclosed the synthesis of
radionuclidic "calixspherands", which are capable of
forming stable complexes with radionuclides such as
 $^{81}Rb^+$. These calixspherands are composed of a
20 calixarene backbone which is conjugated to a *m*-
terphenyl moiety. The *m*-terphenyl moiety is
subsequently derivatized and linked to a low molecular
weight protein (LMWP) which facilitates organ (in this
case, kidney) targeting. The calixspherand-LMWP
25 conjugate thus formed is then complexed with $^{81}Rb^+$ and
used in conjunction with a scintillation detection for
the determination of blood flow in tissue and organs.

Heretofore, the use of calixarene conjugates as
30 MRI or CT imaging agents has not been reported.

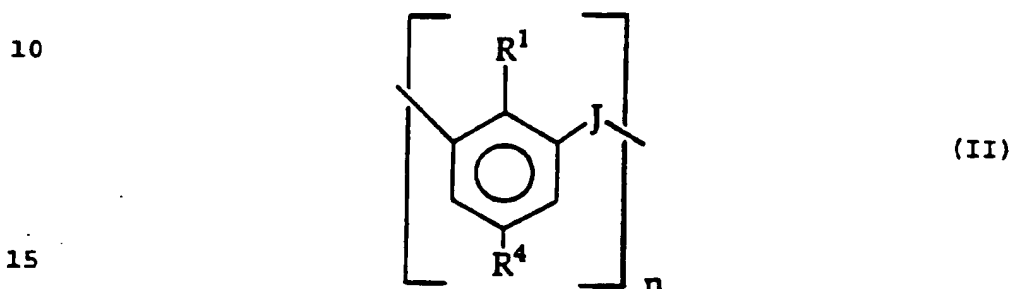
Disclosure of the Invention

The present invention relates to calixarene
conjugates useful for imaging, particularly magnetic
35 resonance imaging (MRI) and computed tomography (CT).

Accordingly, one aspect of the invention relates
to calixarene conjugates comprising: (i) a calixarene

backbone; and (ii) at least one imaging moiety linked thereto. Preferably, at least one imaging moiety is an MR imaging moiety or a CT imaging moiety.

Another aspect of the invention relates to calixarene conjugates of the formula:



wherein at least one of the R^1 and R^4 substituents comprises an imaging moiety, the remaining R^1 and R^4 substituents, if any, are spectator groups, J is an ortho-linker, and n is an integer from 4 to 8.

Yet another aspect of the invention relates to calixarene conjugates useful for CT imaging wherein the imaging moiety comprises two or more iodine atoms.

Still another aspect of the invention relates to calixarene conjugates useful for MRI wherein the imaging moiety comprises at least one of (i) an organic moiety comprising four or more fluorine atoms; (ii) a nitroxyl spin labeled moiety; or (iii) a metal chelate moiety.

Yet another aspect of the invention relates to imaging agent formulations comprising a calixarene conjugate comprising a calixarene backbone and at least one CT or MR imaging moiety linked thereto, and a pharmaceutically acceptable carrier.

Still another aspect of the invention relates to methods of CT and/or MR imaging comprising the steps of (i) administering an effective amount of a calixarene conjugate of the invention; and (ii)

acquiring a CT and/or MR image of the subject while
5 the calixarene conjugate is present in the body.

Brief Description of the Drawings

10

Figure 1 is a flow chart that illustrates a synthetic route for the preparation of a calixarene conjugate having an iodinated CT imaging moiety.

15 Figure 2 is a flow chart that illustrates a synthetic route for the preparation of a calixarene conjugate having a fluorinated MR imaging moiety.

Figure 3 is a flow chart that illustrates a synthetic
20 route for the preparation of a calixarene conjugate having a nitroxyl spin labeled MR imaging moiety.

Figure 4 is a flow chart that illustrates a synthetic
route for the preparation of a calixarene conjugate
25 having a paramagnetic metal chelate MR imaging moiety.

Figure 5 is a flow chart that illustrates a synthetic
route for the preparation of a bifunctional calixarene
conjugate having both a iodinated CT imaging moiety
30 and a fluorinated MR imaging moiety.

Detailed Description of the Invention

The invention relates to imaging agents that
35 enhance diagnostic images generated by magnetic resonance imaging (MRI) and computerized tomography (CT). These agents are comprised of calixarenes which

have been conjugated to one or more imaging moieties
5 to form calixarene conjugate imaging agents.

The terms listed below, as used herein, shall
have the following meaning:

10 Calixarene: Macrocyclic compound comprised of
ortho-linked phenolic units. Also included in the
term "calixarenes" are derivatives of calixarenes such
as those which result from the substitution and/or
derivatization of the -OH in the 1-position.

15 Calix[n]arene: A calixarene wherein n refers to
the number of phenolic units in the macrocycle.

Calixarene Conjugate Imaging Agent: A calixarene
which is conjugated to at least one imaging moiety.

Calixarene Backbone: The calixarene portion of a
20 calixarene conjugate imaging agent.

Imaging Agent: A compound containing at least
one imaging moiety which, when administered to a
subject, alters or enhances a diagnostic image of a
part of the subject.

25 Imaging Moiety: The functional portion of an
imaging agent which contains the chemical entity that
alters or enhances the diagnostic image.

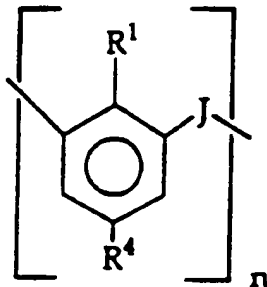
Linker Group: The chemical moiety which serves
to covalently attach one or more imaging moieties to
30 the calixarene.

Ortho-Linker: The linkage between phenolic units
of a calixarene wherein the ortho-carbon of one phenyl
group is linked to the ortho-carbon of the adjacent
phenyl group.

35

A. Calixarene conjugates

The calixarene conjugate imaging agents of the present invention are described by the following formula:



(II)

wherein R¹ is a substituent at the 1-position, R⁴ is a substituent at the 4-position, J is the ortho-linker between adjacent phenyl groups of the calixarene backbone, and n is an integer from 4 to 8, preferably 4, 6 or 8, more preferably 4 or 8, most preferably 8.

At least one of the R¹ or R⁴ groups comprises an imaging moiety. It is contemplated that the imaging moiety portion of the calixarene conjugate imaging agent may comprise an MRI or CT imaging agent. When more than one of the R¹ or R⁴ groups comprise an imaging moiety, the groups may be the same or different, but are preferably the same. Each R¹ and R⁴ group may also contain more than one imaging moiety, in which case the imaging moieties may be the same or different, but are preferably the same.

Those R¹ and R⁴ groups which do not comprise an imaging moiety instead comprise a spectator substituent. The spectator substituent is a pharmacologically acceptable group and may be chosen to enhance synthetic ease, water solubility, ionic charge, neutrality, and the like. The term "pharmacologically acceptable", as used herein,

denotes a substituent which is inactive or innocuous
in vivo. Examples of spectator substituents include,
for example, the -OH group in the 1-position of
simple calixarenes, which may be left unchanged, or
may be derivatized to a different spectator
substituent, such as O-CH₃.

Further examples of spectator substituents
include -H; alkyl (linear, branched, or cyclic) of 1
to 15 carbon atoms, more preferably 1-6 carbon atoms,
yet more preferably methyl; phenyl or substituted
phenyl of 6 to 20 carbon atoms, such as aryl; aralkyl
of 7 to 20 carbon atoms, more preferably substituted
benzyl; alkaryl of 7 to 20 carbon atoms such as
substituted phenyl; -OH; alkoxy of 1 to 10 carbon
atoms, more preferably 1 to 6 carbon atoms, yet more
preferably methoxy; carboxylic acid such as -CO₂H or
-(CH₂)_m-CO₂H, wherein m is 1 to 4; sulfonic acid such
as -SO₃H or -(CH₂)_m-SO₃H; amino acid such as
-NH-(CH₂)_m-CO₂H wherein m is as defined above;
sulfonic acid amine such as -NH-(CH₂)_m-SO₃H, wherein m
is as defined above; ethanolamine groups, such as
-NHCH₂CH₂OH and -N(CH₂CH₂OH)₂; and amide such as
-NHC(=O)R^S, -NR^SC(=O)R^S, -C(=O)NHR^S, or -C(=O)N(R^S)₂,
wherein R^S, which may be the same or different, is
also a pharmacologically acceptable group. The
spectator substituent is preferably -H, alkyl,
alkylsulfonate, alkylcarboxylate (with alkyl of 1-7
carbon atoms), aminoalkylamide (-C(=O)NH(CH₂)_mNH₂,
wherein m is as defined above); and pharmacologically
acceptable salts thereof. See, *inter alia*, Yudelson,
et al., 1993, U.S. Patent No. 5,233,995; Speck et al.,
1993, U.S. Patent No. 5,232,685; Speck et al., 1993,
U.S. Patent No. 5,183,654; McCarthy et al., 1993, U.S.
Patent No. 5,177,261; Willie, 1993, U.S. Patent No.

- 5,204,086; Kneller et al., 1993, U.S. Patent No.
5 5,191,120; Dimo et al., 1984, U.S. Patent No.
4,474,747; and references therein.

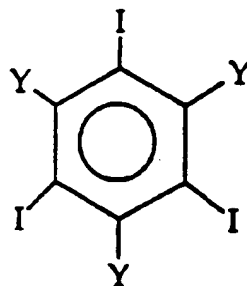
The term "salt" as used herein denotes both
suitable metal ion and organic ion salts. Suitable
10 pharmacologically acceptable salts include metal ions,
for example, alkali and alkaline earth cations,
preferably Na^+ , K^+ , Mg^{+2} , and Ca^{+2} , more preferably
 Na^+ and K^+ , and organic ions, for example, stable
cationic and anionic species such as halide ion,
15 preferably Cl^- or Br^- , N-methylglucamine ("meglumine")
cation, and tris(hydroxymethyl)amino methane ("TRIS")
cation.

The imaging moieties are covalently attached to
20 calixarenes via a linker group. In some instances, it
may be possible to link one or more imaging moieties
directly to a calixarene via a covalent bond, in which
case a linker group or groups is/are not necessary.
Examples of linker groups include, for example, amide
25 $(-\text{NH}-\text{C}(=\text{O})-)$, sulfonamide $(-\text{NH}-\text{S}(=\text{O})_2-)$, thiourea
 $(-\text{NH}-\text{C}(=\text{S})-\text{NH}-)$, urea $(-\text{NH}-\text{C}(=\text{O})-\text{NH}-)$, disulfide
 $(-\text{SS}-)$, thioether $(-\text{S}-)$, amidine $(-\text{NH}-\text{C}(=\text{NR})-)$, and
carbamate $(-\text{NH}-\text{C}(=\text{O})-\text{O}-)$ linkages, and are preferably
amide, sulfonamide, thiourea, and urea linkages.

30 The ortho-linker, J, is a chemical moiety which
covalently joins together the phenyl groups of the
calixarene backbone. Examples of ortho-linkers
include $-(\text{CH}_2)_p-$, $-(\text{C}=\text{O})-$, $-\text{CHR}-$, $-(\text{S}=\text{O})-$, $-(\text{P}=\text{O})-$,
35 preferably $-\text{CH}_2-$, $-(\text{C}=\text{O})-$, and $-\text{CHR}-$, and more
preferably $-\text{CH}_2-$, wherein R is a hydrocarbyl, such as
alkyl of 1 to 4 carbon atoms, preferably methyl, and p
is an integer from 1 to 4, preferably 1 to 2.

CT imaging agents and the imaging moieties they
5 are comprised of are either more or less electron
dense than the tissues or organs being imaged so as to
increase the differentiation therebetween. They are
most typically electron beam opacifiers, also known as
radiopaques, such as bromine or iodine-containing
10 compounds, preferably the latter. CT imaging moieties
preferably comprise two or more iodine atoms, more
preferably three or more iodine atoms. A wide variety
of CT imaging agents are known in the art (see, inter
alia, Radiographic Contrast Agents, R.E. Miller et
15 al., 1977, University Park Press, Baltimore,
Maryland). Iodine-containing compounds suitable as
sources of CT imaging moieties include, for example,
polyiodinated phenyls, more preferably triiodinated
phenyls, of the following general formula:

20

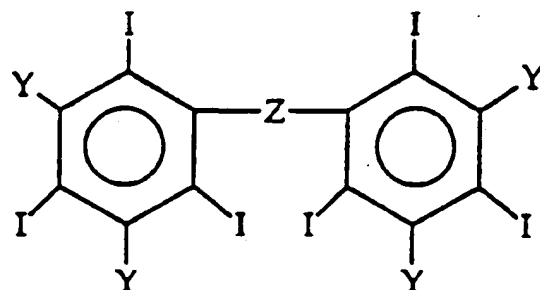


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(III)

30 wherein Y is a pharmacologically acceptable group, as
described above.

Additional iodine-containing compounds suitable
as sources of CT imaging moieties include, for
35 example, polyiodinated phenyl dimers of the following
general formula:



(IV)

5

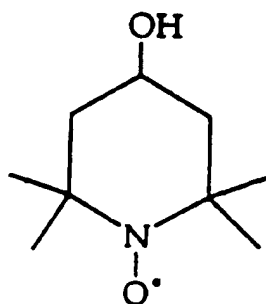
wherein Y is as defined above and Z is a linking
group, including, for example, -NH-C(=O)-
10 $(\text{CH}_2)_q\text{-C(=O)-NH-}$ and $\text{-C(=O)-NH-(CH}_2)_q\text{-NH-C(=O)-}$,
wherein q is 0 to 4, more preferably 0 to 2 (see,
inter alia, Radiographic Contrast Agents, Miller et
al., 1977, *supra*; Kneller et al., 1993, *supra*; Dima et
15 al., 1984, *supra*; and references therein).

MR imaging agents and the imaging moieties they
are comprised of typically are substances that have
magnetic properties which cause the brightening or
20 darkening of a magnetic resonance image. Several
different classes of MR imaging agents/imaging
moieties are known. Among them are fluorine-
containing organic compounds, nitroxyls, and
paramagnetic metal chelates. Fluorine-containing
25 imaging moieties of choice possess more than three
magnetically equivalent fluorine atoms, more
preferably six or more magnetically equivalent
fluorine atoms, most preferably nine or more
magnetically equivalent fluorine atoms. As used
30 herein, the term "magnetically equivalent" denotes
atoms present in a moiety or compound which yield
magnetic resonance signals of a sufficiently similar
frequency that they form a single resonance peak as
detected by typical diagnostic magnetic resonance
35 imaging apparatus (see, for example, Rogers et al.,
1993, U.S. Patent No. 5,234,680).

A variety of fluorine-containing compounds
5 suitable for use as imaging moieties are known in the
art (see, *inter alia*, Rogers et al., U.S. Patent No.
5,116,599, 1992 and 5,234,680, 1993) and include, for
example, compounds possessing the perfluoro-tert-butyl
group, such as $C(CF_3)_3-(CH_2)_r-NH_2$;
10 $C(CF_3)_3-(CH_2)_r-C(=O)X$; $C(CF_3)_3-(CH_2)_r-X$; 3,5-
di(perfluoro-tert-butyl-methyl)benzoyl halide; and 4-
perfluoro-tert-butyl-methylbenzoyl halide; wherein r
is 1 to 5, preferably 2 to 4, and X (halide) is Cl,
Br, or I, preferably Cl or Br. Additional examples of
15 suitable fluorine-containing compounds are those
possessing two or more perfluoromethyl groups, such as
3,5-di(trifluoromethyl)benzoyl halide, wherein halide
is Cl, Br, or I, preferably Cl or Br.

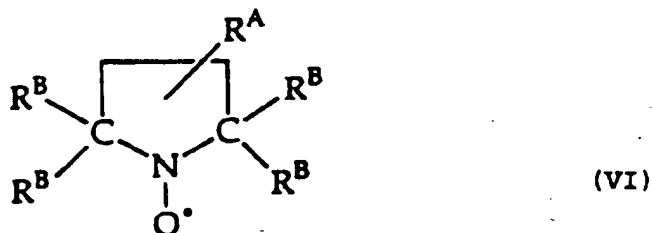
20 Nitroxyl-containing imaging moieties include
nitroxyl spin labels (NSP). Such moieties typically
are organic, possess at least one nitroxyl ($-N-O\cdot$)
free radical, and are paramagnetic by virtue of having
one unpaired electron. Nitroxyl-containing imaging
25 moieties may be derived from the wide variety of
piperidine-based NSP compounds which are known in the
art (see, *inter alia*, Keana, 1989, U.S. Patent No.
4,863,717; Berchadsky et al., 1991, U.S. Patent No.
5,006,663; Leunback, 1990, WO 90/00904; and references
30 therein) and include, for example, the piperidinoxyl
moiety derived from 1,4-dihydroxy-2,2,6,6-
tetramethylpiperidine, as shown below.

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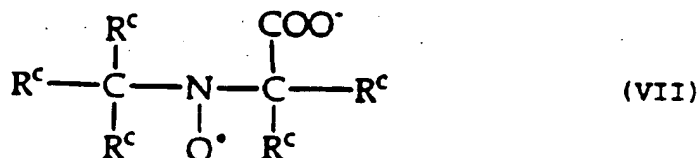


(V)

Additional nitroxyl-containing imaging moieties
 5 may be derived from pyrrole-based NSP compounds which
 are known in the art (see, *inter alia*, Rosen, 1993,
 U.S. Patent No. 5,256,397; Rosen, 1992, U.S. Patent
 No. 5,104,641; Berchadsky et al., 1991, *supra*; Rosen,
 1989, *supra*; and Leunback, 1990, *supra*; and references
 10 therein) and include, for example, 2,2,5,5-
 pyrrolinidyl-oxyl compounds of the following formula,
 wherein R^A is a carboxylalkyl or aminoalkyl group, and
 R^B, which may be the same or different, is an alkyl
 group.



20 Further nitroxyl-containing imaging moieties may
 be derived from *tert*-butyl-based NSP compounds which
 are known in the art (see, *inter alia*, Rosen, 1992,
supra; and references therein) and include, for
 25 example, nitroxides of the following formula, wherein
 R^C, which may be the same or different, is alkyl,
 preferably methyl.



35 Another class of MR imaging moieties useful for
 conjugation to calixarenes is the paramagnetic metal
 chelates. Metal chelate imaging moieties may be
 derived from paramagnetic metal complex MR imaging

agents which are known (see, *inter alia*, Sherry et al., 1992, WO 92/08725, Sherry et al., 1994, U.S. .
5 Patent No. 5,316,757, and references therein). Useful MRI calixarene conjugate imaging agents may be prepared by conjugating a chelating moiety to a calixarene backbone and subsequently forming a
10 chelate-complex between the chelating moiety and a paramagnetic metal. Alternatively, calixarene conjugate imaging agents may be prepared by first forming a paramagnetic metal-complex between a chelating moiety and a paramagnetic metal and
15 subsequently conjugating the paramagnetic metal-complex to a calixarene backbone.

The term "paramagnetic metals" as used herein denotes metal atoms or ions which are paramagnetic by
20 virtue of one or more unpaired electrons, and excludes radioactive metal atoms or ions commonly referred to as radionuclides. Examples of paramagnetic metals used in MR imaging agents of the invention include the paramagnetic transition metals and lanthanides of
25 groups 1b, 2b, 3a, 3b, 4a, 4b, 5b, 6b, 7b, and 8, more preferably those of atomic number 21-31, 39-50, 57-71, and 72-82, yet more preferably Gd, Dy, Cr, Fe, and Mn, still more preferably Gd, Mn, and Fe, and most preferably Gd.

30

The term "chelating moieties" as used herein denotes chemical moieties which are able to form
chelate-complexes with paramagnetic metals. Examples
of linear chelating moieties used in such MR imaging
35 agents include the polyamino polyethylene polyacetic acids (e.g. ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA), triethylene tetraamine hexaacetic acid (TTHA), and

5 tetraethylene pentaamine heptaacetic acid), more preferably DTPA and EDTA. Examples of cyclic chelating moieties used in such imaging agents include polyazamacrocyclic compounds (see, for example, Sherry et al., 1992, 1994, *supra*) such as 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA).

10

Bi-functional calixarene conjugates useful for both MRI and CT are also contemplated. For example, calixarene conjugates may be prepared which have at least two imaging moieties, of which at least one is a CT imaging moiety and at least one is an MR imaging moiety.

15

B. Preparation of Calixarene Conjugates

20 The calixarene conjugates of the invention may be prepared by a number of strategies. One strategy involves first the formation of a calixarene backbone followed by the derivatization of the calixarene backbone to yield the calixarene conjugate. A simple and easily synthesized calixarene (e.g., wherein R^1 is -OH, R^4 is $-C(CH_3)_3$, and J is $-CH_2-$) can be first
25 activated to provide a reactive functional group. An imaging moiety can then, if necessary, be activated to also possess a reactive functional group. The activated calixarene and the activated imaging moiety
30 compound can then be reacted together to yield a calixarene conjugate comprising an imaging moiety attached to a calixarene backbone. The term "reactive functional group" as used herein denotes a chemical group which is capable of reacting with another
35 chemical group, which may also be a "reactive functional group", to form a covalent bond.

For example, an activated calixarene possessing a
5 sulfonyl halide group, such as sulfonyl chloride group
at the 4-position, is reacted with an activated
imaging moiety compound possessing a reactive amino
group, such as N-(2'-aminoethyl)-2,4,6-triiodo-3-
aminobenzamide, to yield a calixarene conjugate
10 possessing a sulfonamide linkage ($-S(=O)_2-NH-$). The
sulfonamide linkage may then be further derivatized,
for example, by using propane-1,3-sultone and base, to
yield a water soluble calixarene conjugate salt. See,
for instance, Example 1 below.

15

A wide variety of pairs of reactive functional
groups may be employed to effect conjugation of the
calixarene with an imaging moiety. Examples of
preferred pairs of reactive functional groups include
20 a sulfonyl halide ($-SO_2X$) and an amino group ($-NH_2$)
which yield a sulfonamide linkage ($-SO_2NH-$); an amino
group ($-NH_2$) and an isothiocyanato group ($-NCS$) which
yield a thiourea linkage ($-NH-C(=S)-NH-$); an amino
group ($-NH_2$) and an active ester group ($-C(=O)OR^*$,
25 wherein R^* is an activating group, such as
succinimidyl or 1-benzotriazolyl, the latter yielding
a water soluble leaving group) or an anhydride
($-C(=O)OC(=O)R$, wherein R is a group such as aryl or
alkyl, which leaves in the acid form when reacted with
30 the amino group) or an acid halide ($-C(=O)X$, wherein X
is halide such as Cl, Br, or I, preferably Cl) which
yield an amide linkage ($-NH-C(=O)-$); and an amino
group ($-NH_2$) and an isocyanato group ($-NCO$) which
yield a urea linkage ($-NH-C(=O)-NH-$).

35

Additional examples of pairs of reactive
functional groups include an amino group ($-NH_2$) and an
amidine ester group ($-C(=NY)OZ$) which yield an amidine

linkage (-NH-C(=NR)-); an amino group (-NH₂) and a
5 haloformate group (-OC(=O)X) which yield a carbamate
linkage (-NH-C(=O)O-); a sulfhydryl group (-SH) and a
haloacetyl group (-C(=O)CH₂X) which yield a
-SCH₂C(=O)- linkage; a sulfhydryl group (-SH) and an
alkyl halide group (-alkyl-X) or an alkyl sulfonate
10 group (-S(=O)₂O-alkyl) which yield a thioether linkage
(-S-); and a sulfhydryl group (-SH) and another
sulfhydryl group (-SH) which yield a disulfide linkage
(-SS-); wherein X (halide) is as defined above, Y is a
pharmacologically acceptable group such as hydrogen or
15 methyl and Z is a group such as aryl or alkyl which
leaves in the alcohol form when reacted with the amino
group.

Included in the term "reactive functional groups"
20 are those functional groups which can be activated by
known methods. For example, active esters (-C(=O)OR^{*},
wherein R^{*} is, as defined above and acid halides
(-C(=O)X, wherein X (halide) is as defined above may
be derived from carboxylic acids.

25 As to which member of the pair of reactive
functional groups is present on either the activated
calixarene or the activated imaging moiety compound,
the choice may be governed by synthetic convenience
30 and ease of purification.

Both the calixarene 1-position and 4-position may
be variously derivatized to yield reactive functional
groups by known methods. For example, the more
35 versatile 4-position may be derivatized to yield
reactive functional groups such as -SO₂Cl, -NH₂, -NO₂,
-SH, -CN, -COOH, and the like. The -OH group in the
1- position of simple calixarenes may be used as a

reactive functional group, or it may be derivatized to
5 yield, *inter alia*, reactive functional groups such as
-O(CH₂)_nCO₂R and O(CH₂)_nSO₃M where M is a metal such
as sodium or potassium and R is an alkyl or
substituted alkyl or M.

10 Many suitable activated imaging moieties are
known in the art. For example, the NSP compound 4-
amino-2,2,6,6,-tetramethyl-1-piperidinoxyl (also known
as 4-amino-TEMPO) and the iodinated compound N-(2'-
aminoethyl)-2,4,6-triiodo-3-aminobenzamide both
15 possess amino groups which may react with sulfonyl
halide groups. Similarly, the fluorinated compound
C(CF₃)₃CH₂CH₂Br possesses an alkyl halide group which
may react with a phenolic -OH group.

20 Activated imaging moieties may also be obtained
by derivatizing known compounds, including, for
example, known imaging agents, to yield compounds
possessing a reactive functional group. For example,
a chelating agent useful in the formation of
25 paramagnetic metal complexes, such as DTPA, may be
derivatized by known methods to yield a reactive
isothiocyanato group (-NCS) (see Example 4 below).
Similarly, the chelating agent DOTA (1,4,7,10-tetra-
azacyclododecane-1,4,7,10-tetraacetic acid) may be
30 derivatized by known methods, for example, to yield
the 2-(p-isothiocyanatobenzyl) derivative.

In some instances, one or both of the calixarene
and imaging moiety compound need not be activated to
35 permit conjugation. For example, calixarene
conjugates may be formed by reaction of simple
calixarenes wherein R¹ is -OH and R⁴ is -H with
imaging moiety compounds such as

triiodobenzoylchloride (to yield a CT imaging agent)
5 or $(\text{CF}_3)_3\text{C}(\text{CH}_2)_s\text{C}(=\text{O})\text{X}$ (to yield an MR imaging agent
wherein s is about 1 to 5 and X (halide) is Cl, Br, or
I). In the latter example, the phenyl ring of the
calixarene with the $-\text{H}$ in the R^4 position reacts with
the acyl (Friedel-Crafts reaction), resulting in the
10 formation of a covalent bond.

An alternative strategy for the formation of
calixarene conjugates involves the derivatization of a
calixarene backbone. For example, a simple calixarene
15 which has been derivatized to have a haloalkyl group
(e.g. $-\text{CH}_2\text{Cl}$) at the 4-position may be reacted under
conditions described by Rogers et al. (1993, U.S.
Patent No. 5,234,680; perfluoroisobutylene with cesium
fluoride in monoglyme) to yield a calixarene conjugate
20 containing the MR imaging moiety $-\text{C}(\text{CF}_3)_3$.

Yet another alternative strategy for the
formation of calixarene conjugates involves first
derivatizing pre-calixarene monomers followed by the
25 formation of a calixarene structure. For example,
perfluoro-tert-butylphenol monomers may be prepared by
reacting p-fluoro-nitrobenzene with $\text{Cs}^+\text{C}(\text{CF}_3)_3^-$ in
monoglyme to yield nitrobenzene, followed by reduction
of the nitro group to an amino group, diazotization of
30 the amino group to yield a diazonium salt, and
hydrolysis to yield a hydroxy group. In the same
manner that 4-tert-butylphenol is reacted with
formaldehyde to yield a simple calixarene, 4-
perfluoro-tert-butylphenol may be reacted with
35 formaldehyde to yield a calixarene conjugate wherein
the imaging moiety is perfluoro-tert-butyl ($-\text{C}(\text{CF}_3)_3$)
in the 4-position.

5 The molecular weight of the resulting calixarene conjugate, and therefore the nature and degree of the imaging moieties and spectator substituents, may be selected to optimize in vivo behavior. For example, Guerbet et al. (1993, WO 93/10824) discuss the beneficial aspects of high molecular weight CT imaging agents for the blood pool. Imaging agent molecular weights of at least about 3,000 g/mol, more preferably at least about 5,000 g/mol, yet more preferably at least about 6,500 g/mol are desirable. Although there remains contention in the art as to the existence of an upper limit for preferred blood pool imaging agent molecular weights, such an upper limit may be less than about 15,000 g/mol, more preferably less than about 13,000 g/mol, yet more preferably less than about 11,500 g/mol.

20 Note that the molecular weight of the simple calixarene wherein R^1 is -OH, R^4 is $-C(CH_3)_3$, J is $-CH_2-$, and n is 8, is approximately 1296 g/mol. The approximate molecular weights of the imaging agents described in Examples 1, 2, 3, 4, and 5 are, respectively, Compound (8): 7065 g/mol, Compound (13): 3858 g/mol, Compound (15): 3796 g/mol, Compound (18): 7290 g/mol, and Compound (22): 9032 g/mol.

30 The calixarene conjugates of the invention may or may not be water-soluble, but preferably are water-soluble. The water-solubility of the calixarene conjugates may be influenced by the choice of the imaging moiety and/or spectator substituent. In particular, the imaging moiety and/or spectator substituent may be chosen to further comprise one or more chemical groups which influence water solubility of the calixarene conjugate.

Water solubility of the calixarene conjugates may
5 be increased by incorporating chemical groups which
are ionic or neutral. Conversely, incorporating
chemical groups, such as hydrophobic groups, will
reduce the overall water-solubility of the calixarene
conjugate. Additionally, many nitroxyl containing CT
10 imaging moieties are hydrophobic, and imaging agents
comprising such imaging moieties will be less water-
soluble. Also, neutral hydrophobic spectator groups
such as, for example, the hydrocarbyl groups (e.g.,
alkyl, aryl, aralkyl, and alkaryl) described above may
15 reduce overall water-solubility.

The number and choice of chemical groups may
further be selected to influence the overall
neutrality or charge of the calixarene conjugate.
20 Although ionic species are often more water-soluble,
neutral water-soluble species often afford more
favorable osmolalities. Examples of such chemical
groups include carboxylic acid, sulfonic acid, amino
acid, sulfonic acid amine, ethanolamine, and amide.

25 The calixarene conjugates of the present
invention may optionally be further conjugated to one
or more targeting moieties, wherein the targeting
moiety permits or enhances tissue or organ
30 specificity, including, for example, kidney, liver, or
tumor specificity. For example, further conjugation
to yield a high molecular weight water soluble
calixarene conjugate may permit targeting properties
suitable for blood pool imaging. Also, further
35 conjugation to a low molecular weight protein may
permit kidney specificity, whereas further conjugation
to metalloporphyrins may permit tumor (such as breast
tumor) specificity. Imaging agent formulations

comprising microemulsions of water-insoluble
5 calixarene conjugates may permit liver specificity.

C. Methods of Imaging

The methods of CT and MRI are well known in the
10 art. See, inter alia, The Contrast Media Manual,
(1992, R.W. Katzberg, Williams and Wilkins, Baltimore,
Maryland), especially chapter 6 ("Contrast Media Use
in Computed Tomography") and chapter 13 ("Magnetic
Resonance Contrast Agents").

15 Typically, an effective amount of an imaging
agent formulation comprising the calixarene conjugate
and pharmaceutically acceptable carrier is
administered to the patient, and the patient, or a
20 portion of the patient, is imaged. The term
"effective amount", as used herein, denotes a non-
toxic amount sufficient to enhance or alter the CT or
MRI image obtained, more particularly, an amount which
permits better visualization of the organs and/or
25 tissues being imaged. Preferably the patient is an
animal; more preferably, the patient is a mammal; most
preferably the patient is a human.

The imaging agents of the present invention may
30 be variously administered by any suitable route,
including, for example, orally, for imaging of the
upper gastrointestinal tract; rectally, for imaging of
the lower gastrointestinal tract including the colon;
nasally, for imaging of the nasal and communicating
35 passages; vaginal, for imaging of the fallopian tubes
and communicating passages; parenteral (including
subcutaneous, intramuscular, intravenous, intradermal
and pulmonary), for imaging of internal organs,

tissues, tumors, and the like. It will be appreciated
5 that the preferred route will vary with the organs or
tissues to be imaged. Preferred routes of
administration include parenteral and oral, more
preferably intravenous.

10 While it is possible for the imaging agent to be
administered alone, it is preferable to present it as
a pharmaceutical formulation comprising at least one
imaging agent compound, together with one or more
15 pharmaceutically acceptable carriers, such as diluents
or excipients which may include, for example, fillers,
extenders, wetting agents, disintegrants, surface-
active agents, or lubricants, depending on the nature
and mode of administration and the dosage forms. Each
20 carrier must be "acceptable" in the sense of being
compatible with the other ingredients of the
formulation and not injurious to the patient. The
pharmaceutical formulation may optionally include
other diagnostic or therapeutic agents. Techniques
and formulations may be found, for example, in
25 Remington's Pharmaceutical Sciences, Mack Publishing
Co., Easton, PA. (latest edition).

Formulations of the present invention suitable
for oral administration may be presented as a solution
30 or suspension in an aqueous or non-aqueous liquid; or
as an oil-in-water liquid emulsion; or a water-in-oil
liquid emulsion. Alternatively, formulations can be
administered as capsules, cachets or tablets, each
containing a predetermined amount of the imaging
35 agent; powder; granules; or paste.

Formulations suitable for parenteral
administration include aqueous and non-aqueous

isotonic sterile injection solutions which may contain
5 anti-oxidants, buffers, bacteriostats and solutes
which render the formulation isotonic with the blood
of the intended recipient; and aqueous and non-aqueous
sterile suspensions which may include suspending
agents and thickening agents, and liposomes or other
10 microparticulate systems which are designed to target
the compound to blood components or one or more
tissues or organs.

The formulations may be presented in unit-dose or
15 multi-dose sealed containers, for example, ampules and
vials, and may be stored in a freeze-dried
(lyophilized) condition requiring only the addition of
the sterile liquid carrier, for example water, for
injections immediately prior to use. Extemporaneous
20 injection solutions and suspensions may be prepared
from sterile powders, granules or tablets.

It should be understood that in addition to the
ingredients particularly mentioned above, the
25 formulations of this invention may include other
agents conventional in the art having regard to the
type of formulation in question, for example, those
suitable for oral administration may include such
further agents as sweeteners, thickeners and flavoring
30 agents.

Compounds of the formula of the present invention
may also be presented for use in the form of
veterinary formulations, which may be prepared, for
35 example, by methods that are conventional in the art.

For CT, dosages may be conveniently calculated as
milligrams of halide, for example, iodine per kilogram

of patient (abbreviated as mg(I)/kg). For parenteral
5 administration, typical dosage volumes for an average
human adult are 100-300 mL, preferably about 200 mL,
with formulation concentrations of about 100-300
mg(I)/mL, preferably 200 mg(I)/mL. An average human
patient of weight 70 kg may therefore receive about
10 571 mg(I)/kg for an overall dosage of about 40 g(I).

For MRI contrast agents, dosages will depend on
the spin density, flow (diffusion and perfusion),
susceptibility, and relaxivity (T1 and T2) of the
15 imaging agent formulation. For MRI, dosages may be
conveniently calculated as millimoles of contrast
agent per kilogram of patient (abbreviated as
mmol(A)/kg). For example, for parenteral
administration, typical dosages may be 0.01 to
20 1 mmol(A)/kg.

Rates of administration are known in the art.
Typical rates of administration are about 0.5 to 5 mL
of formulation per second, more preferably about 1-3
25 mL/s. Imaging may begin before or after commencing
administration, continue during administration, and
may continue after administration. It will be
appreciated that dosages, dosage volumes, formulation
concentrations, rates of administration, and imaging
30 protocols will be individualized to the particular
patient and the examination sought, and may be
determined by an experienced practitioner. Guidelines
for selecting such parameters are known in the art
(see, *inter alia*, Katzberg, 1992, *supra*).

35

D. Examples

5

Example 1Synthesis of a Calixarene Conjugate Useful for CT

10 The synthesis of compound (8) is described below and is shown schematically in Figure 1. Compound numbers in parentheses refer to the structures shown in the Figures.

15 Compound (2): octa(1-hydroxy)octa(4-tert-butyl)
calix[8]arene

Compound (2) was prepared according to the method described by Munch et al. (Organic Synthesis, 1989, 68:243-245.) A slurry of *p*-tert-butylphenol (Compound
20 (1), 100.0 g, 0.67 mol), paraformaldehyde (35 g, 1.1 mol) and 2 mL of 10 N sodium hydroxide in xylene (600 mL) was placed in a 2 L, round bottomed, three-necked flask fitted with a Dean-Stark water collector and a mechanical stirrer. The slurry was heated in an
25 atmosphere of argon to reflux with stirring. After 1 hr, a white precipitate started to separate. The reaction mixture was heated for 8 hr. The mixture was then cooled to room temperature and the precipitate filtered to remove unreacted components and
30 by-products. The crude product was washed, in succession, with 400 mL portions of toluene, ether, acetone, and water and then dried under reduced pressure. The product was dissolved in chloroform and crystallized. The resultant crystals were separated
35 by filtration and dried. Yield = 70.5 g, 67%; purity in TLC (SiO₂), hexane/dichloromethane (8:2), R_f = 0.55; NMR (CDCl₃) = 1.23 (s, 72H t-butyl), 3.55 and

4.42 (2d, 16H, CH₂), 7.19 (s, 16H, aromatic), 9.53 (s,
5 8H, OH), δ ppm.

Compound (3): octa(1-hydroxy)calix[8]arene

10 Compound (3) was prepared according to the method
described by Gutsche et al. (Tetrahedron, 1986,
42:1633-1640). A slurry of compound (2) (20.0 g,
0.015 mol), phenol (12.0 g, 0.124 mol) and anhydrous
aluminum chloride (25 g, 0.186 mol) in toluene
15 (300 mL) was stirred at room temperature for 1 hr in
an argon atmosphere. The mixture was poured into
500 mL of water at 4°C and stirred for 1 hr. The
insoluble slurry thus formed (top layer), which
contained the product with phenol, was separated from
20 the aqueous (bottom) layer and the toluene was removed
in a rotary evaporator. The slurry was then washed in
succession with 100 mL acetone, 0.1 N hydrochloric
acid, methanol, chloroform, acetone and ether, and
then filtered and dried under reduced pressure. Yield
25 = 12.2 g, 98%; NMR (pyridine) = 4.25 (s, br, 16H,
CH₂), 6.85 (t, 8H, aromatic), 7.19 (s, br, 16H,
aromatic), 9.34 (s, br, 8H, OH) δ ppm.

Compound (4): octasodium octa(1-hydroxy)octa(4-
30 sulfonato)calix[8]arene

Compound (3) (4.2 g, 0.0047 mol) was stirred with
concentrated sulfuric acid (35 mL) and heated to 60°C
for 4 hr. The insoluble product was filtered through
35 a glass filter and the solid was dissolved in 120 mL
water. The solution was neutralized with excess
barium carbonate (10.0 g) to pH 6-7 and was
subsequently filtered. The filtrate was adjusted to

pH 8-9 with a calculated amount of sodium carbonate
5 (1.4 g). The aqueous solution was lyophilized to
produce a colorless powder (3.8 g). The powder was
redissolved in water (10 mL) and diluted with equal
amounts of ethanol. The product in the sodium salt
form was filtered and dried. Yield = 3.8 g, 70%; NMR
10 (D_2O) = 3.9 (s, 16H, CH_2), 7.45 (s, 16H, aromatic) δ
ppm.

Compound (5): octasodium octa(1-methoxy)octa(4-
sulfonato)calix[8]arene

15
Compound (5) was prepared according to the method
described by Shinkai et al. (J. Amer. Chem. Soc.,
1986, 108:2409-2416). Compound (4) (3.2 g, 0.002 mol)
was dissolved in sodium hydroxide solution (15 mL,
20 2.24 g, 0.056 mol) and dimethylsulfoxide (50 mL) was
added to the mixture. Iodomethane (8.4 g, 0.059 mol)
in dimethylsulfoxide (10 mL) was added and the
solution was heated to 50-55°C for 24 hr. The mixture
was cooled and diluted with ethanol (200 mL). The
25 solid product was filtered and dried. The solid was
subsequently dissolved in 10 mL water and diluted with
15 mL ethanol. The precipitated solids were filtered
and dried. This procedure was repeated three times to
remove the excess sodium iodide. The product formed
30 pale yellow crystals. Yield = 1.82 g, 60%; NMR (D_2O)
= 3.27 (s, 24H, OCH_3), 4.08 (s, 16H, CH_2), 7.50 (s,
16H, aromatic) δ ppm.

Compound (6): octa(1-methoxy)octa(4-chlorosulfonyl)
35 calix[8]arene

Compound (6) was prepared according to the method
described by Shinkai et al. (Bull. Chem. Soc. Jpn.,

1990, 63:1272-1274). Compound (5) (1.5 g, 8 mmol) was
5 refluxed with thionyl chloride (15 mL) in the presence
of a few drops of dimethylformamide. After 4 hr, the
reaction mixture was cooled and poured into ice-water.
The precipitate was recovered by filtration and
extracted into chloroform. The chloroform layer was
10 dried with Na₂SO₄ and filtered. Removal of the
chloroform followed by crystallization produced pale
yellow crystals. Yield = 64%, mp = 286°C (dec); NMR
(D₂O) = 3.62 (s, 24H, OCH₃), 4.21 (s, 16H, CH₂), 7.68
(s, 16H, aromatic) δ ppm.

15

Compound (7):

The reagent, N-(2'-aminoethyl)-3-amino-2,4,6,-
triiodobenzamide, is prepared from 3-amino-2,4,6-
20 triiodobenzoic acid and ethylene diamine via the
carboxylic acid chloride. Compound (7) is then
prepared by a method analogous to the one described by
Shinkai et al. (1990, *supra*) by reaction of Compound
(6) (0.1 g, 0.05 mmol) with 2 equivalents of the
25 reagent N-(2'-aminoethyl)-3-amino-2,4,6,-
triiodobenzamide (0.51 g, 0.89 mmol) in 5 mL pyridine.

Compound (8):

30 Compound (7) in dimethylsulfoxide is treated with
excess sodium hydride and propane-1,3-sultone to
obtain Compound (8) using a method analogous to that
described by Shinkai et al. (J. Chem. Soc. Perkin
Trans. I, 1989, 2039-2045).

35

Example 25 Synthesis of a Calixarene Conjugate Useful for ^{19}F MRI

The synthesis of compound (13) is described below and is shown schematically in Figure 2. Compound numbers in parentheses refer to the structures shown
10 in the Figures.

15 Compound (9): octa(1-methoxy)calix[8]arene

Compound (9) was prepared according to the method described by Gutsche et al. (1986, *supra*). A mixture containing compound (3) (4.5 g, 0.0053 mol), sodium
20 hydride (80% in oil, 7.2 g, 0.188 mol) in tetrahydrofuran (200 mL) was prepared and reacted at room temperature to form the sodium salt. Then dimethylsulfate (26.6 g, 0.211 mol) in DMF (50 mL) was added. The resultant mixture was heated at 70°C for
25 20 hr. It was then cooled and the excess sodium hydride was decomposed with methanol (25 mL). Methanol, tetrahydrofuran and DMF were removed under reduced pressure. The residue thus formed was washed with water (150 mL) and methanol (100 mL) to yield the
30 crude product which was then passed through a silica gel column (45 g). The product was eluted with a mixture of hexane and ethyl acetate (1:1) followed by dichloromethane to obtain pale yellow crystals. Recrystallization from methanol and chloroform yielded
35 3.61 g, 71%. NMR (CDCl_3) = 3.5 (s, 24H, OCH_3), 4.0 (s, 16H, CH_2), 6.8 (s, 8H aromatic) δ ppm.

Compound (10): octa(1-methoxy)octa(4-chloromethyl)
5 calix[8]arene

Compound (10) is prepared according to the method described by Aimi et al. (Tetrahedron, 1989, 45:2177-2182). Compound (9) in chloroform solution is reacted
10 with stannic chloride at -10°C for 50 min. The reaction mixture is poured into water and after extraction and removal of chloroform, the product is crystallized to produce compound (9) in pure form.

15 Compound (11): octa(1-methoxy)octa{4-[(perfluoro-tert-butyl)methyl]}calix[8]arene

Compound (11) is prepared according to the method described by Rogers et al. (1993, U.S. Patent No. 5,234,680). Compound (10) in monoglyme (CH₃O-CH₂CH₂-OCH₃) is reacted with perfluoroisobutylene and cesium fluoride at room temperature for 20 hr and is worked-up by filtering off the cesium bromide and removing
20 the monoglyme solvent. The product is extracted in chloroform and the solvent is dried and then removed in a rotary evaporator. The residue is recrystallized to yield compound (11).
25

30 Compound (12): octa(1-hydroxy)octa{4-[(perfluoro-tert-butyl)methyl]}calix[8]arene

Compound (12) is prepared according to the method described by McOmie et al. (Tetrahedron, 1968, 24:2289-2292), for de-methylation of methyl ethers.
35 Compound (11) is dissolved in dichloromethane and reacted with excess boron tribromide at -60°C to -40°C for 3 to 4 hr. The mixture is poured into dilute

hydrochloric acid and the dichloromethane layer is
5 separated and the solvent is dried and then removed.
The residue is purified by crystallization and/or
chromatography to yield compound (12).

Compound (13): octasodium octa{1-(3-
10 sulfonatopropoxy)}octa{4-[(perfluoro-tert-
butyl)methyl]} calix[8]arene

Compound (13) is prepared by the method described
by Shinkai et al. (1989, *supra*). Compound (12) in
15 tetrahydrofuran is treated with excess sodium hydride
and propane-1,3-sultone to obtain compound (13).

Example 3

Synthesis of a Calixarene Conjugate Comprising an
20 Organic Paramagnetic Group Useful for MRI

The synthesis of compound (15) is described below
and is shown schematically in Figure 3. Compound
numbers in parentheses refer to the structures shown
25 in the Figures.

Compound (14):

The compound (14) is prepared according to
30 similar procedures described earlier by Shinkai et al.
(1990, *supra*). Compound (6) is reacted with excess 4-
amino-TEMPO (4-amino-2,2,6,6,-tetramethyl-1-
piperidinoxyl, Aldrich Chemical Company, Milwaukee,
Wisconsin) in chloroform. Compound (14) is isolated
35 and purified by crystallization.

Compound (15):

5

Compound (14) in tetrahydrofuran is reacted with excess sodium hydride and propane-1,3-sultone as described by Shinkai et al. (1990, *supra*) to obtain the compound (15) as a water soluble sodium salt.

10

Example 4

Synthesis of Calixarene Conjugates Comprising a Paramagnetic Metal Complex Moiety Useful for MRI

15 The synthesis of compound (18) is described below and is shown schematically in Figure 4. Compound numbers in parentheses refer to the structures shown in the Figures.

20 Compound (16):

Compound (6) is treated with N-(tert-butoxycarbonyl)ethylenediamine in a suitable solvent at room temperature as described by Essian et al. (J. Med. Chem., 1988, 31:898-891) to yield compound (16).

25

Compound (17): octa(1-methoxy)octa(4-[N-(2'-amino)ethyl)sulfonamido]calix[8]arene

30

Compound (16) is converted to Compound (17) in free-base form by reacting with trifluoroacetic acid in a manner analogous to that described by Betebenner et al. (Bioconjugate. Chem., 1991, 2:117-123).

35

Compound (18): DTPA Conjugate

5

The reagent, 3-(4-isothiocyanatobenzyl)-6 methyl-diethylene-tetraaminepentaacetic acid, is prepared as described by Brechbiel et al. (Bioconjugate Chemistry, 1990, 1(1):59-68) and reacted with compound (17) in dichloromethane at room temperature as described by Weiner et al. (Mag. Res. Med., 1994, 31:1-8) to give compound (18).

10

Example 5

15 Synthesis of Bifunctional Calixarene Coniugates Useful For Both MRI and CT

The synthesis of compound (22) is described below and is shown schematically in Figure 5. Compound numbers in parentheses refer to the structures shown in the Figures.

20

Compound (19): octa{1-[3-(perfluoro-tert-butyl)propoxy]} calix[8]arene

25

Compound (19) is prepared by a method analogous to the one described by Shinkai et al. (1990, *supra*). Compound (3) is converted to the sodium salt with excess sodium hydride in a solvent such as tetrahydrofuran or dimethylformamide and then reacted 1-bromo-3-(perfluoro-tert-butyl)propane to furnish Compound (19) which is purified by crystallization or chromatography. 1-bromo-3-(perfluoro-tert-butyl)propane is prepared by reacting perfluoroisobutylene and cesium fluoride in a solvent like monoglyme with excess dibromopropane as described by Rogers et al. (1993, U.S. Patent No. 5,234,680).

30

35

Compound (20): octa{1-[3-(perfluoro-tert-
5 butyl)propoxy]} octa(4-chlorosulfonyl)calix[8]arene

Compound (20) is prepared by reacting
Compound (19) with excess chlorosulfonic acid in a
solvent like chloroform. After stirring the mixture
10 at room temperature for 6-8 hours, the reaction
mixture is poured into ice water and the product
obtained from the organic layer after suitable workup
and crystallization.

15 Compounds (21) and (22):

Compounds (21) and (22) are prepared from
compound (20) by reactions analogous to ones described
above for the preparation of compounds (7) and (8),
20 respectively.

Example 6

In Vivo CT Imaging

25 Imaging agents for CT are prepared as described
in Examples 1 and 5 and suspended in a
pharmaceutically acceptable carrier. CT imaging is
carried out by standard procedures using commercially
30 available equipment. The x-ray beam energy is
typically 120 KeV although dual energy beam systems
are available. X-ray CT is an inherently two-
dimensional imaging method that acquires transaxial
images of any region of the human body, provided that
35 region is located within the x-ray beam-detector
gantry. Conventional CT scanners use fixed parameters
for slice thickness; the in-plane resolution can be
adjusted within pre-determined parameters set by the

5 manufacturer (e.g., 256 X 256 or 512 pixel resolution and scan time, which is a function of the resolution.) Spiral or helical scanning CT units allow for more options of slice thickness and typically have shorter scan times (about 1 second/slice).

10 The subject to be imaged is placed on a CT patient platform ("couch"). An initial alignment using the positioning system of the scanner and external anatomic reference points in the subject is done. A "scout" image is done to determine if the
15 subject is properly located within the CT gantry; if not, the subject is repositioned by remotely controlling the travel of the patient platform to obtain the desired location. This is repeated until desired alignment is achieved.

20 Typically, a series of precontrast images are obtained. Following administration of the contrast agent, the CT examination is performed while the contrast agent is present in the region being imaged.
25 Imaging dosages are calculated as described in Example 8.

Example 7

In Vivo MRI

30 Imaging agents for MRI are prepared as described in Examples 2 to 5 and suspended in a pharmaceutically acceptable carrier. Proton (^1H) and/or fluorine (^{19}F) imaging are carried out using standard procedures and commercially available equipment. Proton imaging can
35 be performed with the following parameters:
Repetition time (TR) = 1 second, echo time (TE) = 18 milliseconds, image data matrix = 128 X 128, number of excitations (NEX) = 2, field of view (FOV) = 128 nm,

and slice thickness = 2.5 or 5.0. Fluorine imaging
5 can be performed with the following parameters: TR = 1
second, TE = 18 milliseconds, image data matrix = 64 X
64, NEX = 32, FOV = 128 nm.

Proton and fluorine MRI are done before and after
10 administration of the contrast agent. When using
fluorine-containing imaging agents, such as those
described in examples 2 and 5, proton MRI is used to
provide anatomic markers for assessment of the
fluorine images. Imaging dosages are calculated as
15 described in Example 8.

Example 8

Imaging Dosage Calculations

Imaging dosages will depend on the solubility of
20 the imaging agent, the route of administration, the
carrier vehicle, the site to be imaged and the method
of imaging. Described in Table 1 are three exemplary
imaging dosages for a 70 kg human subject using the
compounds of Examples 1 to 4.

25

30

35

Table 1

| Imaging Agent | Molecular Weight, g/mol | Dosage A (Amount to Administer) | Dosage B (Amount to Administer) | Dosage C (Amount to Administer) |
|--------------------------|----------------------------------|--|--|--|
| Example 1 Compound 8 | 7065 (43.14% I) | 100 mg I/Kg (16.25 g) | 200 mg I/Kg (32.48 g) | 300 mg I/Kg (48.72 g) |
| Example 2 Compound 13 | 3858 (35.5% ^{19}F) | 100 mg ^{19}F /Kg (19.7 g) | 250 mg ^{19}F /Kg (49.3 g) | 500 mg ^{19}F /Kg (98.7 g) |
| Example 3 Compound 15 | 3796 (8 spins*/ molecule) | 1.5 mmol (1 spin per molecule)/Kg (49.9 g) | Not Determined | Not Determined |
| Example 4 Compound 18 | 7290 (1258 mg Gd/mmol) | 0.1 mmol Gd/Kg (6.38 g) | Not Determined | Not Determined |

* A "spin" is the result of the presence of one unpaired electron. Thus, a molecule such as compound 15, of Example 8, which contains 8 nitroxyl free radicals per molecule is said to contain 8 spins per molecule.

CLAIMS

5

1. A calixarene conjugate comprising:
(a) a calixarene backbone; and
(b) at least one imaging moiety linked thereto.

10

2. The calixarene conjugate of claim 1 wherein the imaging moiety is an MR imaging moiety.

3. The calixarene conjugate of claim 1 wherein the imaging moiety is a CT imaging moiety.

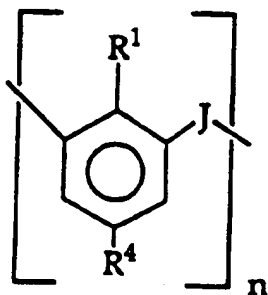
15

4. The calixarene conjugate of claim 1 wherein the conjugate has at least two imaging moieties, wherein at least one moiety is a CT imaging moiety and at least one moiety is a MR imaging moiety.

20

5. A calixarene conjugate of the formula:

25



30

- wherein at least one of the R^1 and R^4 substituents comprises an imaging moiety, the remaining R^1 and R^4 substituents, if any, are spectator groups, J is an ortho-linker, and n is an integer from 4 to 8.

35

6. The calixarene conjugate of claim 5, wherein said ortho-linker is $-CH_2-$.

- 5 7. The calixarene conjugate of claim 5, wherein n = 4.
8. The calixarene conjugate of claim 5, wherein all the R¹ groups comprise an imaging moiety.
- 10 9. The calixarene conjugate of claim 5, wherein all the R⁴ groups comprise an imaging moiety.
10. The calixarene conjugate of claim 5, wherein said imaging moiety is a CT imaging moiety.
- 15 11. The calixarene conjugate of claim 10, wherein said CT imaging moiety comprises two or more iodine atoms.
- 20 12. The calixarene conjugate of claim 10, wherein said CT imaging moiety comprises three or more iodine atoms.
- 25 13. The calixarene conjugate of claim 10, wherein said CT imaging moiety comprises a tri-iodinated phenyl group.
- 30 14. The calixarene conjugate of claim 5, wherein said imaging moiety is an MR imaging moiety.
15. The calixarene conjugate of claim 14, wherein said MR imaging moiety comprises four or more fluorine atoms.
- 35 16. The calixarene conjugate of claim 15, wherein said fluorine atoms are present as part of -CF₃ groups.

17. The calixarene conjugate of claim 16, wherein
5 said fluorine atoms are present as part of one or more
-C(CF₃)₃ groups.
18. The calixarene conjugate of claim 14, wherein
10 said MR imaging moiety comprises at least one nitroxyl
spin labeled moiety.
19. The calixarene conjugate of claim 18, wherein
said nitroxyl spin labeled moiety comprises a
piperidinoxyl moiety.
- 15 20. The calixarene conjugate of claim 14, wherein
said MR imaging moiety comprises at least one metal
chelate moiety.
- 20 21. The calixarene conjugate of claim 20, where said
metal chelate moiety comprises a DTPA moiety.
22. The calixarene conjugate of claim 20, where said
metal chelate moiety comprises a Gd(III) ion.
- 25 23. An imaging agent formulation comprising:
(a) a calixarene conjugate comprising:
a calixarene backbone, and
at least one CT imaging moiety linked thereto;
30 and
(b) a pharmaceutically acceptable carrier.
24. An imaging agent formulation comprising:
(a) a calixarene conjugate comprising:
35 a calixarene backbone, and
at least one MR imaging moiety linked thereto;
and
(b) a pharmaceutically acceptable carrier.

25. A method of CT imaging comprising:

5 (a) administering an effective amount of a calixarene conjugate having at least one CT imaging moiety to a patient; and

10 (b) acquiring a CT image of at least a portion of the patient while the calixarene conjugate is present in the patient.

26. A method of MRI comprising:

15 (a) administering an effective amount of a calixarene conjugate having at least one MR imaging moiety to a patient; and

(b) acquiring an MR image of at least a portion of the patient while the calixarene conjugate is present in the patient.

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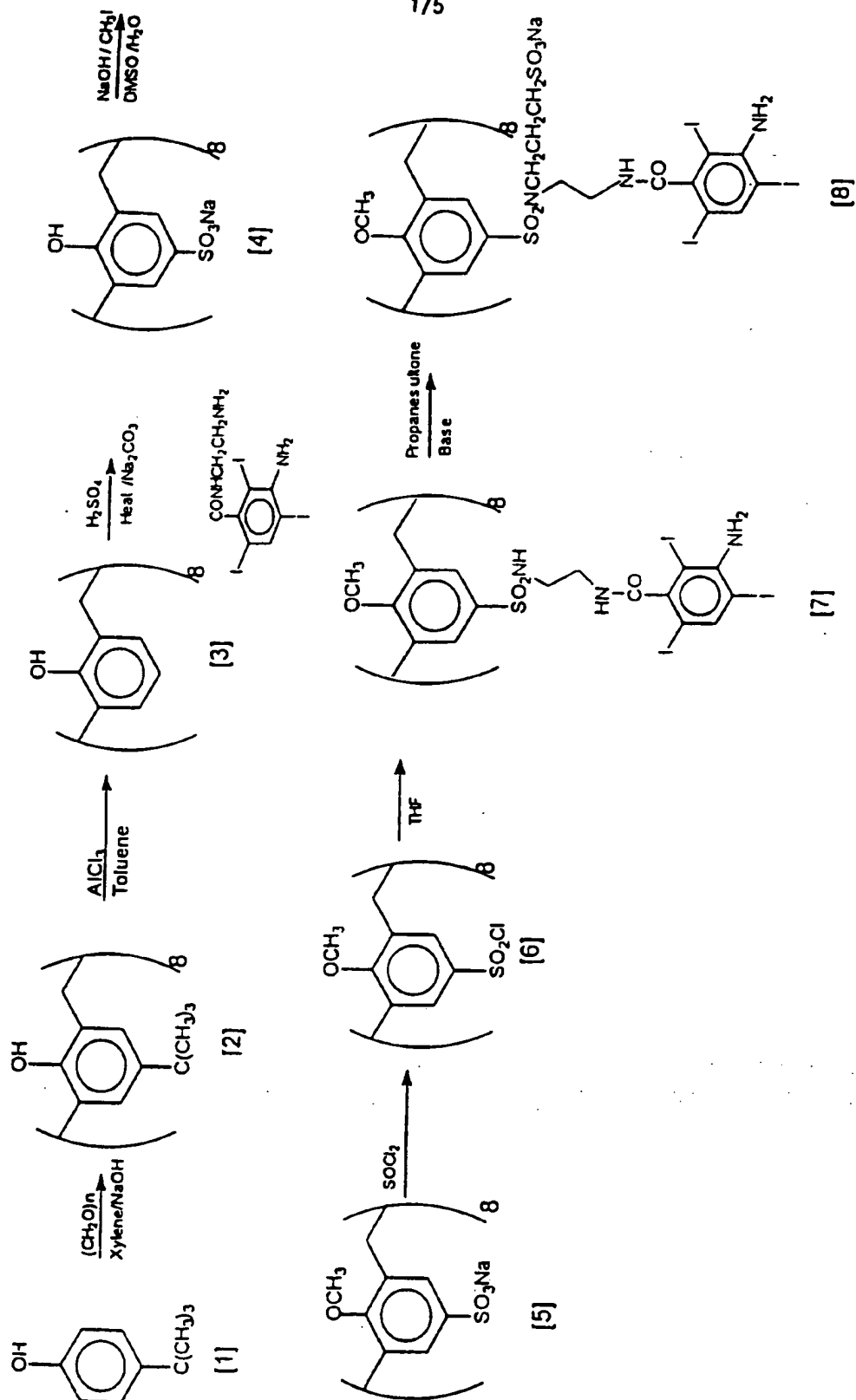


Figure 1

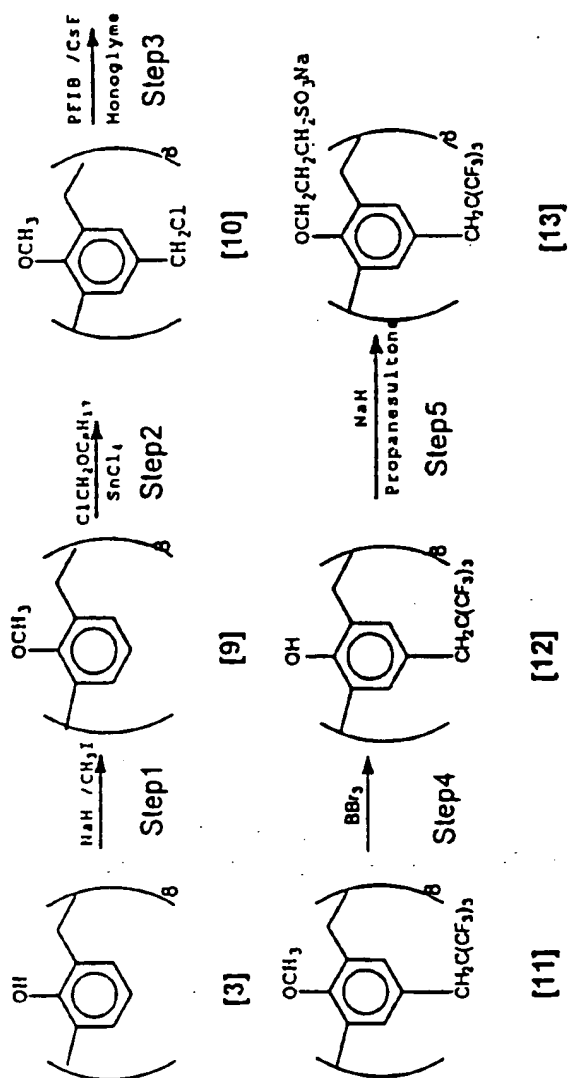


Figure 2

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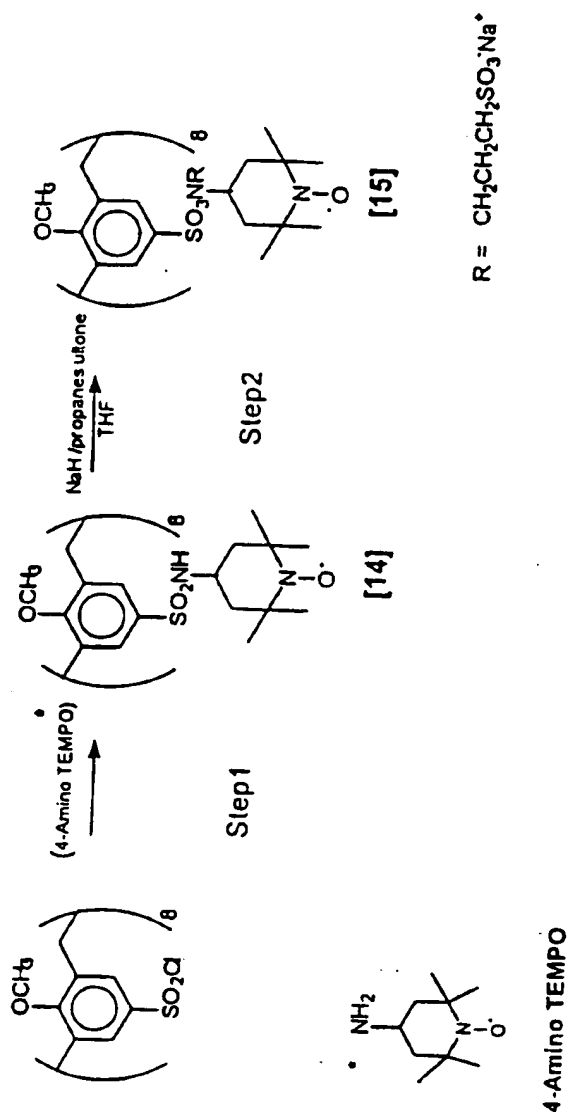


Figure 3

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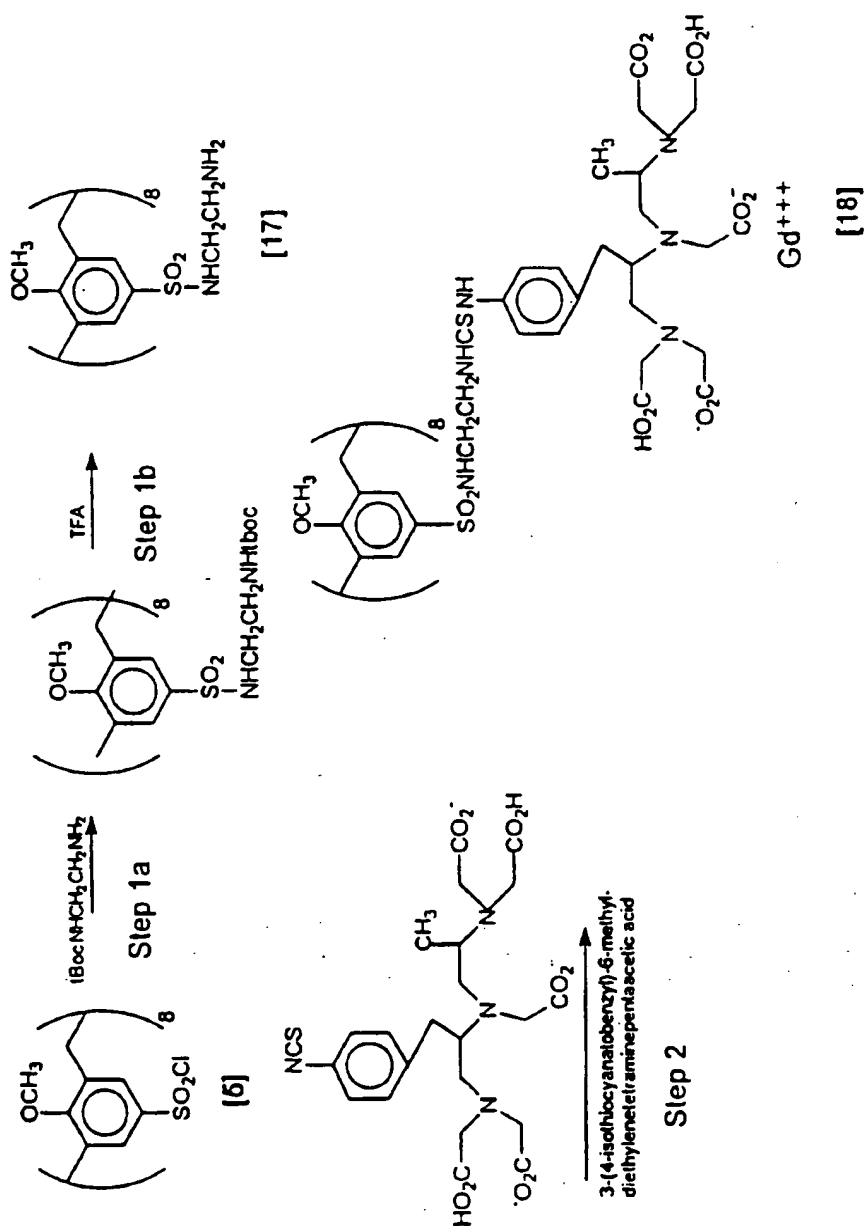


Figure 4

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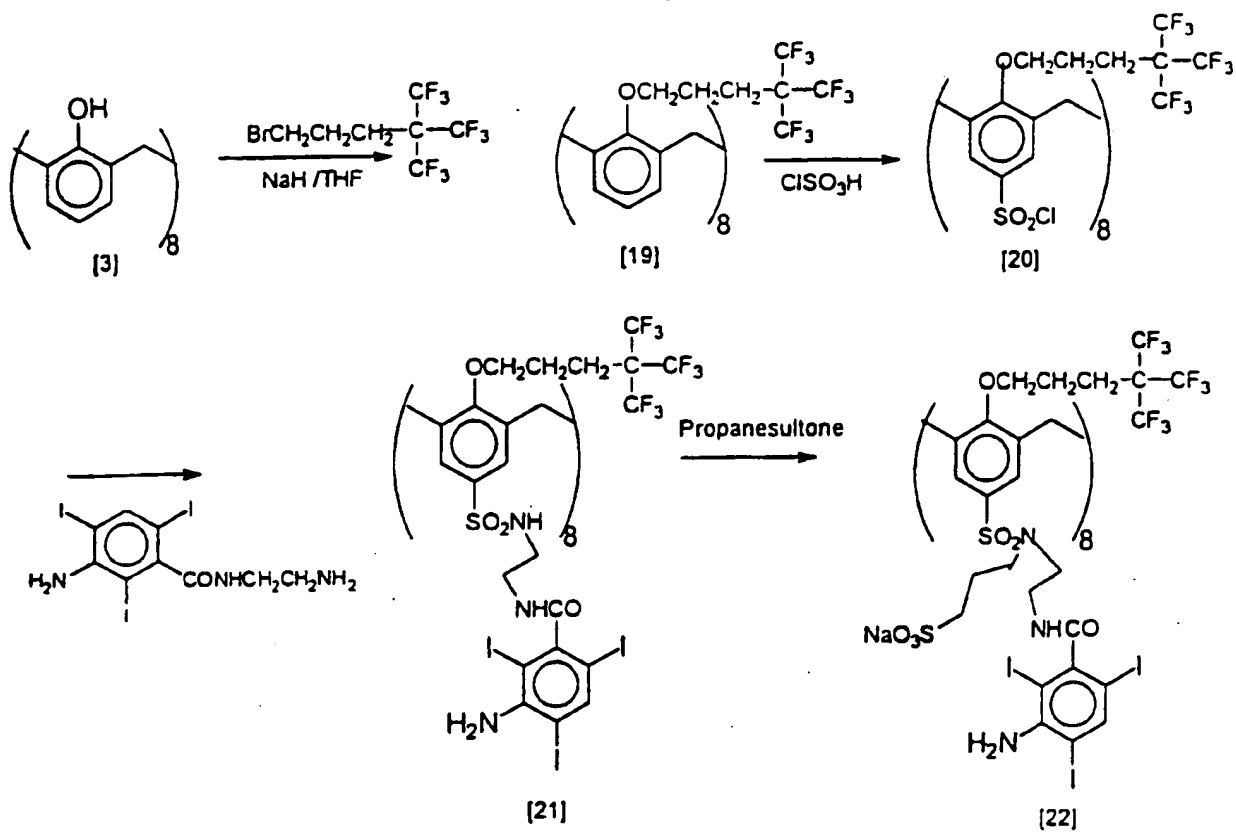


Fig 5